

10/001688

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<u>L4</u>	L3 and (low\$5 near5 temperature41)	0	<u>L4</u>
<u>L3</u>	11 and ((oligonucleotide\$1 or nucleic acid\$1) near5 solid)	26	<u>L3</u>
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- ☐ 1. [6512105](#). 30 Jun 00; 28 Jan 03. Methods for making oligonucleotide probes for the detection and/or quantitation of non-viral organisms. Hogan; James John, et al. 536/24.3; 435/6 436/501 536/23.1 536/24.31 536/24.32 536/25.3 702/20. C07H021/00 C12Q001/68 G06F019/00.
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- ☐ 2. [6342349](#). 21 Jul 98; 29 Jan 02. Optical disk-based assay devices and methods. Virtanen; Jorma. 435/6; 435/91.1 435/91.2 536/22.1 536/23.1 536/24.3 536/25.3. C12Q001/68 C12P019/34 C07H019/00 C07H021/00 C07H021/02.
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- ☐ 3. [6150517](#). 30 May 95; 21 Nov 00. Methods for making oligonucleotide probes for the detection and/or quantitation of non-viral organisms. Hogan; James John, et al. 536/25.3; 435/6 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C07H021/00.
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- ☐ 4. [5994059](#). 30 May 95; 30 Nov 99. Nucleic acid probes and methods for detecting Streptomyces enterococci. Hogan; James John, et al. 435/6; 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☐ 5. [5958679](#). 30 May 95; 28 Sep 99. Nucleic acid probes and methods for detecting Enterobacter cloacae. Hogan; James John, et al. 435/6; 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☐ 6. [5840488](#). 06 Jun 95; 24 Nov 98. Nucleic acid probes for detection and/or quantitation of non-viral organisms. Hogan; James John. 435/6; 435/5 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33 536/25.3. C12Q001/68.
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- ☐ 7. [5827651](#). 30 May 95; 27 Oct 98. Nucleic acid probes and methods for detecting fungi. Hogan; James John, et al. 435/6; 435/5 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☐ 8. [5714321](#). 30 May 95; 03 Feb 98. Nucleic acid probes and methods for detecting salmonella. Hogan; James John. 435/6; 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68 C07H021/00 C07H021/02 C07H021/04.
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- ☐ 9. [5693469](#). 30 May 95; 02 Dec 97. Nucleic acid probes and methods for detecting Escherichia coli. Hogan; James John. 435/6; 435/810 435/91.1 435/91.2 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68 C07H021/00 C07H021/02 C07H021/04.
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- ☐ 10. [5693468](#). 30 May 95; 02 Dec 97. Nucleic acid probes and methods for detecting chlamydia trachomatis. Hogan; James John, et al. 435/6; 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☒ 11. [5691149](#). 30 May 95; 25 Nov 97. Nucleic acid probes and method for detecting *Mycoplasma pneumoniae*. Hogan; James John, et al. 435/6; 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☒ 12. [5679520](#). 30 May 95; 21 Oct 97. Nucleic acid probes and methods for detecting eubacteria. Hogan; James John, et al. 435/6; 435/5 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☒ 13. [5677129](#). 30 May 95; 14 Oct 97. Nucleic acid probes and methods for detecting legionella. Hogan; James John, et al. 435/6; 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☒ 14. [5677128](#). 30 May 95; 14 Oct 97. Nucleic acid probes and methods for detecting mycobacterium. Hogan; James John, et al. 435/6; 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☒ 15. [5677127](#). 30 May 95; 14 Oct 97. Nucleic acid probes and methods for detecting group I pseudomonas. Hogan; James John, et al. 435/6; 435/810 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☒ 16. [5674684](#). 30 May 95; 07 Oct 97. Nucleic acid probes and methods for detecting campylobacters. Hogan; James John, et al. 435/6; 435/810 435/91.2 436/501 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.
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- ☒ 17. [5593841](#). 05 Dec 94; 14 Jan 97. Nucleic acid probes for detection and/or quantitation of non-viral organisms. Hogan; James, et al. 435/6; 435/810 436/501 536/22.1 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68 C07H021/04.
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- ☒ 18. [5547842](#). 06 Sep 94; 20 Aug 96. Nucleic acid probes for detection and/or quantitation of non-viral organisms. Hogan; James, et al. 435/6; 435/5 435/810 435/91.1 435/91.2 436/501 536/22.1 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68 C07H021/04.
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- ☒ 19. [5541308](#). 22 Feb 94; 30 Jul 96. Nucleic acid probes for detection and/or quantitation of non-viral organisms. Hogan; James J., et al. 536/23.1; 435/6 435/810 435/91.1 435/91.2 436/501 436/63 536/22.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C07H021/04 C12Q001/68.
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- ☒ 20. [5482834](#). 19 Jan 93; 09 Jan 96. Evaluation of nucleic acids in a biological sample hybridization in a solution of chaotropic salt solubilized cells. Gillespie; David H.. 435/6; 435/174 435/179 435/810 435/820 536/24.3. C12Q001/68 C12N011/00 C12N015/00 C07H021/00.
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L6: Entry 4 of 20

File: USPT

Nov 30, 1999

DOCUMENT-IDENTIFIER: US 5994059 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Nucleic acid probes and methods for detecting Streptomyces enterococci

Brief Summary Text (6):

Broadly, there are two basic nucleic acid hybridization procedures. In one, known as "in solution" hybridization, both a "probe" nucleic acid sequence and nucleic acid molecules from a test sample are free in solution. In the other method, the sample nucleic acid is usually immobilized on a solid support and the probe sequence is free in solution.

Detailed Description Text (47):

As to nucleic acid concentration, it is known that the rate of hybridization is proportional to the concentration of the two interacting nucleic acid species. Thus, the presence of compounds such as dextran and dextran sulphate are thought to increase the local concentration of nucleic acid species and thereby result in an increased rate of hybridization. Other agents which will result in increased rates of hybridization are specified in U.S. application Ser. No. 627,795, entitled "Accelerated Nucleic Acid Reassociation Method", filed Jul. 5, 1984, Continuation-in-Part thereof, Serial No. (net yet assigned), filed Jun. 4, 1987, and U.S. application Ser. No. 816,711, entitled "Accelerated Nucleic Acid Reassociation Method", filed Jan. 7, 1986, both of which are incorporated by reference. (U.S. application Ser. No. 07/644,879, which is a continuation of U.S. application Ser. No. 816,711, issued as U.S. Pat. No. 5,132,207, on Jul. 21, 1992). On the other hand, chemical reagents which disrupt hydrogen bonds such as formamide, urea, DMSO, and alcohols will increase the stringency of hybridization.

Detailed Description Text (50):

In one embodiment of the DNA/rRNA hybridization assay invention, a labelled probe and bacterial target nucleic acids are reacted in solution. rRNA may be released from bacterial cells by the sonic disruption method described in Murphy, K. A. et al., U.S. application Ser. No. 841,860, entitled "Method for Releasing RNA and DNA From Cells", filed Mar. 20, 1986, which is; incorporated herein by reference. (U.S. application Ser. No. 07/711,114, which is a continuation of U.S. application Ser. No. 07/298,765, which is a continuation of U.S. application Ser. No. 06/841,860, issued as U.S. Pat. No. 5,374,522, on Jan. 20, 1994). Other known methods for disrupting cells include the use of enzymes, osmotic shock, chemical treatment, and vortexing with glass beads. Following or concurrent with the release of rRNA, labelled probe may be added in the presence of accelerating agents and incubated at the optimal hybridization temperature for a period of time necessary to achieve significant reaction. Following this incubation period, hydroxyapatite may be added to the reaction mixture to separate the probe/rRNA hybrids from the non-hybridized probe molecules. The hydroxyapatite pellet is washed, recentrifuged and hybrids detected by means according to the label used.

Detailed Description Text (187):

Diagnosis of Campylobacter enteritis is currently dependent upon growth and isolation of the organism in culture, followed by a number of biochemical tests. Optimum growth of campylobacters requires special conditions such as low oxygen tension and high temperature (42.degree. C.). No single set of conditions is recommended for isolation of all Campylobacter species.

Detailed Description Text (205):

The sequence is 35 bases in length and has a Tm of 72.degree. C. It is capable of hybridizing in the region corresponding to bases 825-860 of E. coli 16s rRNA. To demonstrate the reactivity and specificity of the probe, it was used in a hybridization assay with purified RNA or RNA released from cells. A suspension

containing at least  $10^7$  cells in 2% sodium dodecyl sulfate was vortexed in the presence of glass beads. 0.1 ml of suspension was mixed with 0.1 ml of hybridization buffer (0.96M sodium phosphate, pH 6.8, 0.002M EDTA, 0.002M EGTA) and incubated at 65.degree. C. for 2 hours. After incubation, 5 ml of 2% hydroxyapatite, 0.12M sodium phosphate pH 6.8, 0.02% sodium dodecyl sulfate was added and the mixture was incubated at 65.degree. C. for 10 minutes. The sample was centrifuged and the supernatant removed. Five ml of wash solution (0.12M phosphate buffer, pH 6.8, 0.02% sodium dodecyl sulfate) was added and the samples were vortexed, centrifuged, and the supernatant removed. The amount of radioactivity bound to the hydroxyapatite was determined by scintillation counting. Table 43 shows that the probe reacts well with *S. faecium*, *S. faecalis*, and *S. avium*, and does not react with other closely related organisms.

Detailed Description Text (259):

One application of the invention is to detect bacteria in urine (bacteriuria). To demonstrate the reactivity and specificity of the probes for bacteria found in urine, they were used in hybridization assays.  $^{32}$ P-end-labeled or  $^{125}$ I-labeled oligonucleotide probes were mixed with RNA released from cells by standard methods (e.g, the sonic disruption techniques described in Murphy et al., U.S. Pat. No. 5,374,522, detergent with glass beads, or enzymatic lysis). Probe was mixed with RNA in 0.48M sodium phosphate, pH 6.8, 1 mM EDTA, 1 mM EGTA, 1% sodium dodecyl sulfate (0.2 ml final volume) and hybridized at 60.degree. C. for 2 hours. Five ml of 2% hydroxyapatite, 0.12M sodium phosphate pH 6.8, 0.02% sodium dodecyl sulfate was added and the mixture incubated at 60.degree. C. for 10 minutes. The mixture was centrifuged and the supernatant removed. Five ml of wash solution (0.12M sodium phosphate, pH 6.8, 0.02% sodium dodecyl sulfate) was added and the sample was mixed, centrifuged and the supernatant removed. The amount of radioactivity bound to the hydroxyapatite was determined by scintillation counting. Tables 55-68 demonstrate the specificity of these probes and show that a combination of probes could be used to detect all bacteria which have been tested.

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L1 3 HYBRIDIZ#####(10A)(UREA OR ACETAMIDE)(10A) TEMPERATURE#

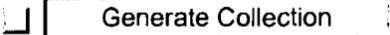
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sucrose-Na citrate can be used as a reliable routine technique. Large  
amts. of intact specific RNA mols. can be purified from DNA-RNA hybrids  
formed in these conditions.  
AN 1971:38292 CAPLUS  
DN 74:38292  
TI DNA-RNA **hybridization** at low **temperature** in the  
presence of **urea**  
AU Kourilsky, Philippe; Manteuil, S.; Zamansky, Marc H.; Gros, Francois  
CS Inst. Biol. Mol., Fac. Sci., Paris, Fr.  
SO Biochemical and Biophysical Research Communications (1970), 41(4), 1080-7  
CODEN: BBRC9; ISSN: 0006-291X  
DT Journal  
LA English  
TI DNA-RNA **hybridization** at low **temperature** in the  
presence of **urea**

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L2: Entry 3 of 7

File: USPT

Feb 27, 2001

DOCUMENT-IDENTIFIER: US 6194146 B1

TITLE: Situ and in vitro hybridization method and buffer

Brief Summary Text (3):

By reference to blot hybridization technology, conventional FISH technology is dependent upon formamide chemistry for DNA denaturation, hybridization buffers, and post-hybridization washes. (Cremer T., Landegent J., Brueckner A., Scholl H P, Schardin M, Hager H D, Devilee P. Pearson P. van der Ploeg M. (1986), "Detection of chromosome aberrations in the human interphase nucleus by visualization of specific target DNAs with radioactive and non-radioactive in situ hybridization techniques: diagnosis of trisomy 18 with probe L.84," Hum. Genet 74:346-352; Pinkel D, Straume T. Gray J W. (1986) "Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization," Proc Natl Acad Sci USA 83 :2934-2938). The entire procedure includes multiple steps for sample denaturation, hybridization and post-hybridization washes. This process often takes hours to yield a fluorescent label with a signal intensity sufficient for routine chromosome enumeration. Although some recent improvements of formamide-based FISH have been introduced, for example the use of co-denaturation of probes and sample and the elimination of formamide in the post-hybridization washes (Abati A, Sanford J. Fetsch P. Marincola F. Wolman S. (1995) "Fluorescence in situ hybridization (FISH): a user's guide to optimal preparation of cytologic specimens," Diagnostic Cytopathology 13:5:486-492), the chemistry of the hybridization reaction is still based on formamide. Known disadvantages of formamide are that it reduces the kinetics of hybridization (Kourilsky Ph. Leidner J. Tremblay. 1971 "DNA--DNA hybridization on filters at low temperature in the presence of formamide or urea, Biochimie 53:1111-1114), oxidizes easily, and is a known teratogen.

Detailed Description Text (4):

16 .mu.g/ml of Aprogenex XY probe in Solution G from International Application WO 96/31626 (20% glycerol (v/v), 10% dextran sulfate (w/v) and 0.9% NaCl (w/v)) was added to a glass slide and covered with a circular coverslip. The sample slide was then heated at 100.degree. C. for 1.5 min to denature the chromosomal DNA, then hybridized at 42.degree. C. for 30 min followed by washing in accordance with the Aprogenex APROPROBE PLUS wash protocol incorporated by reference. The resulting sample was then counter-stained with DAPI and analyzed for bound fluorescence signal. It was determined that the hybridization efficiency was approximately 97.4% with moderate bound signal (scored as 2-3 on a scale of 1 to 5).

Detailed Description Text (8):

16 .mu.g/ml of Aprogenex XY probe in Solution G from International Application WO 96/31626 (20% glycerol (v/v), 10% dextran sulfate (w/v) and 0.9% NaCl (w/v)) and 100 mM DTT was added to a glass slide and covered with a circular coverslip. The sample slide was then heated at 100.degree. C. for 1.5 min to denature the chromosomal DNA, then hybridized at 42.degree. C. for 30 min followed by washing in Saline Wash A for 3 min at 42.degree. C., followed by four washes in Saline Wash B, with each wash being for 30 sec at 42.degree. C. The resulting sample was then counter-stained with DAPI and analyzed for bound fluorescence signal and duration of signal. It was determined that the signal was bound and, when compared to a control sample, without DTT, only the sample processed with the DTT had a signal which did not quickly fade. To determine whether the presence of DTT drove hybridization, subsequent experiments were conducted using a hybridization buffer without glycerol, but with only dextran sulfate, saline and DTT, and it was found that the absence of the glycerol component from the hybridization buffer did not drive hybridization of the synthetic oligonucleotide probes. Further experiments tested whether DTT, with only glycerol and saline, and without dextran sulfate drove hybridization. It was found that the absence of dextran sulfate from the hybridization medium, in the presence of DTT, did drive hybridization of the synthetic oligonucleotide clone probes.

Detailed Description Text (18):

16 .mu.g/ml of Arogenex XY probe in G-buffer (20% glycerol (v/v), 10% dextran sulfate (w/v) and 0.9% NaCl (w/v)) was added to a glass slide and covered with a circular coverslip. The sample slide was then heated at 100.degree. C. for 1.5 min to denature the chromosomal DNA, then hybridized at 42.degree. C. for 30 min followed by washing in accordance with the post-hybridization wash protocol described hereinafter. The resulting sample was then counter-stained with DAPI and analyzed for bound fluorescence signal. It was determined that the hybridization efficiency was approximately 97.4% with moderate bound signal (scored as 2-3 on a scale of 1 to 4).